2 FACTORS INFLUENCING STUDY DESIGN

This section discusses some initial considerations for planning a study of headwater streams. This section is not intended to cover all possible issues when preparing a study or assessment. Rather, general options and some unique considerations for headwater streams are discussed.

Clearly stated objectives (and associated hypotheses) are important to any scientific study and should be decided before moving forward. The objectives should set the initial stage for what and how much will be measured. Therefore, the spatial and temporal scales (sampling resolution) and scope (range or extent of the study) should be determined by the data needed to meet the objectives or test the hypotheses. Logistical and economic constraints also influence the scale and scope of studies. Norris et al. (1992) point out that the objectives of most studies fall into two general categories: 1) determining values at a single location and time; and 2) comparing values from multiple locations or time periods. In the first case the goal is to provide an accurate estimate (e.g., total density), whereas the second focuses on comparing the difference of values between locations or time periods. Downes et al. (2002) identified four general objectives for assessment studies: 1) assess the ecological state of ecosystems; 2) determine if regulated criteria have been exceeded; 3) detect and quantify impacts generated by anthropogenic disturbance(s); and 4) assess the effectiveness of restoration projects. In any case, the objectives should guide the design, implementation, and analysis of the study.

Field sampling designs

After identifying the specific objectives, decisions are made regarding the study design (i.e., how, what, when and where to sample). There are two major categories for study designs: comparative and manipulative. Comparative (also called measurative) studies have location or time period as the primary treatment(s) being investigated, where the treatment exists without the intervention of the scientists. An example of a comparative study is comparing biological and physiochemical measures among streams with different land uses or an intensity gradient of a land practice. The primary treatment of manipulative (or experimental) studies is an intervention or perturbation by the investigators. An example of a manipulative study is measuring the biological characteristics in one set of streams where large woody debris has been removed by the investigators and in another where large woody debris is left intact. Manipulative studies generally offer more control over the independent variables (and therefore greater potential to identify cause-effect relationships) than comparative studies. On the other hand, comparative studies typically offer greater realism and generality than manipulative studies. The main effects (or treatment differences) and associated variation of effects over the study duration of comparative studies are directly relevant to the systems studied. Investigators designing experimental studies should strive to apply realistic manipulations (i.e., relevant to real world situations) to experimental units. Both categories have merits and limitations that should be considered when planning a study (see Diamond 1986 for a detailed discussion).

Spatial and temporal scales of a study should match the objectives and be relevant to the organisms and environments studied. Gotelli and Ellison (2004) identified two aspects of spatial scale that should be addressed when designing a study: the grain (size of the smallest unit of study) and the extent (total area encompassed by all units in the study). Investigators need to efficiently balance the size of the grain and extent of study with logistics and cost to effectively achieve the scope of the objectives. Temporal scale includes the time needed to collect a sample, the frequency of sampling, and the duration of the study. Hierarchical or nested designs can be used to identify variation associated with different spatial scales, and repeated measures designs assess interaction among sampling periods and treatments. Stratification of sampling by habitat type can account for variation that would otherwise be considered in the error.

A critical aspect of a field study is the sample size needed to effectively test a hypothesis or to provide an acceptable level of confidence around estimates of resource condition. Often the emphasis for condition surveys is to estimate the proportion of a resource among classes of condition (e.g., Diaz-Ramos et al. 1996). Condition classes reflect categories of ecological integrity and are measured with indicators representing various physical and biological parameters. Thresholds separating condition classes are typically set by regulatory standards. The formula for estimating the standard error for a proportion is:

$$\hat{\sigma}_p = \sqrt{\frac{p(1-p)}{n}}$$

where p is the proportion of a population representative of a class and n is the total population size (i.e., sample size). By assuming a proportion that results in the largest estimate of the standard error of the proportion (p =0.5), one can visualize that standard error decreases asymptotically with increasing sample size (Figure 2-1).

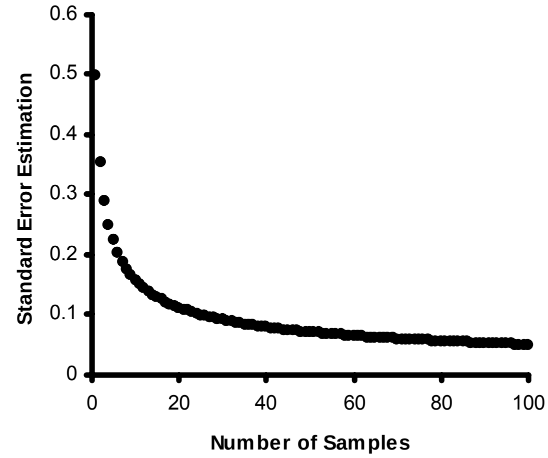

Figure 2-1 Relationship between sample size and standard error estimations assuming proportions are equal among populations.

Therefore, confidence around estimates also increases with higher sample size, but investigators need to balance sampling cost and acceptable level of confidence when designing surveys.

In hypothesis testing, power analysis can be useful for determining the appropriate number of replicates to provide sufficient statistical power for an expected effect size (the detectable difference between treatments) and natural variation (Peterman 1990, Fairweather 1991, Foster 2001). Statistical power measures the probability of correctly rejecting the null hypothesis when in fact it is false (converse of the probability of Type II error). Power is generally described as:

$$Power \propto \frac{ES \cdot \alpha \cdot \sqrt{n}}{s}$$

where ES is effect size, α is the a priori significance level (Type I error probability), n is the sample size, and s is the standard deviation among replicate units. This relationship indicates that for a given effect size and level of variability, power increases with higher study unit replication; however, with that in mind, increasing sample size can enable detection of very small effects that may not be ecologically significant. Larger effect sizes are more likely to be detected than smaller ones with the same sample size and level of variability. The actual formulae for calculating power or deriving appropriate sample size or minimum detectable effect size will vary with statistical test and test statistic (see Cohen 1988, Zar 1998). Effect size may be derived from previous studies, regulatory thresholds, or convention (e.g., order of magnitude). Expected variation can be taken from the literature or pilot studies. An appropriate a priori level of statistical power will vary depending upon the objectives of the study. For example, failing to detect an environmental impact where one exists (i.e., Type II error) may have greater consequences than detecting an impact that does not exist (Type I error), therefore greater power may be desired to protect against a Type II error (see Peterman 1990, Di Stefano 2003). Frequently, cost (time and money) is a critical factor governing sample size. Mapping the study beforehand (estimating time and costs) will help determine the feasibility of the study design. Designing an effective study is balancing effect size, sample size, and cost to meet the study objective.

Randomization should be used whenever feasible to ensure unbiased data collection. Random or probabilistic site selection produces a representative sample of the population(s) targeted under the study objectives, so that results can be more confidently extrapolated to the overall population from which the selected sites were randomly chosen. In contrast, targeted sampling focuses the effort toward a specific problem. The difficulty with randomized site selection is the *a priori* knowledge of the entire population of possible sites or sampling points within the bounds of the study objective. If the scope of the objectives is narrow and the population is known (e.g., water bodies within Central Park), probabilistic sampling is more feasible compared to broader scales where the population is uncertain (e.g., spring seeps of Kentucky). The scope of a study will be narrowed under most circumstances because of the inability to account for the entire population of potential sites. Time of data collection is rarely randomly selected because of the stochastic nature of streams; however, seasonal sampling is usually desired. Index periods are typically determined by the logistics of sampling and the life history of targeted biota.

There are practical difficulties associated with large scale experiments, including the need for a large number of independent replicates to overcome natural variability among replicate study units. A study design that is increasingly used in stream research is the Before/After and Control/Impact (BACI; Stewart-Oaten et al. 1986, Carpenter et al. 1989, Downes et al. 2002). In this design, one or more control sites and one or more impact sites are simultaneously sampled multiple times, both before and after the manipulation to the impact site(s). The difference in parameters measured between the control and impact at each time period represents a replicate unit for the Before and After treatments. Underwood (1991, 1992) strongly advocated the incorporation multiple randomly selected control sites in the design to overcome the possibility that the control and impact sites may have naturally different trends in the measured parameters. Further issues and concerns about BACI designs are reviewed by Smith et al. 1993, Osenberg et al. 1994, and Downes et al. 2002. An in-depth discussion of specific statistical designs is outside the scope of this manual. A few relevant texts for ecological studies include: Clarke and Warwick (2001), Gotelli and Ellison (2004), Scheiner and Gurevitch (1993), Quinn and Keough (2002), and Underwood (1997).

Special considerations for headwater streams
Headwater streams are narrower, shallower, have higher drainage density, and are more likely to dry than larger streams and rivers. Their position in the network also makes many headwater streams more responsive to precipitation, so lag time is shorter between precipitation and peak discharge. Notable exceptions to this are spring-fed streams, where deep and more stable groundwater discharge can dominate the hydrologic regime. Depending upon the geographic location, headwater streams may have higher gradients and therefore the repeating habitat units are typically more closely spaced than wadable streams. Reach lengths for ecological assessment are typically scaled to the channel width (e.g., Barbour et al. 1999, Lazorchak et al. 1998, Moulton et al. 2002). Following this convention, reach lengths of headwaters are shorter than those needed for larger perennial streams and rivers. Multiple reaches or longer reaches may be required for studies using multiple indicators or assessment approaches (i.e. amphibian surveys, tracer additions, etc.). If multiple reaches are used, they should be as close as possible given the sampling or logistical limitations. They should have similar channel dimensions and levels of permanence, avoiding influences by intervening tributary confluences. Higher drainage density affords the opportunity to have nearby replicate streams for studies, but also may result in frequent discontinuities (e.g., abrupt changes in substrate size) at tributary confluences (Rice et al. 2001, Brenda et al. 2004). Unique sampling methods are often required for headwaters because the low flows prevent use of many conventional sampling devices. For example, core samples are preferred for headwater invertebrate sampling rather than Surber or other net samplers that require sufficient flow to carry dislodged debris into the net. Estimates of flow permanence are critical and may be the master variable influencing headwater communities. Measures of channel dimension and substrate size may provide critical insight into the typical flow regime or degree of permanence at a site and should be included in any headwater assessment.

Time of year for sampling is critical in temporary headwaters because precipitation and evapotranspiration has a relatively strong influence on stream discharge. Historic hydrological data are rare for headwater streams because most gauges are positioned on wadeable streams and large rivers. Discharge data from downstream gauges can provide an integrated measure of precipitation and evapotranspiration for a basin. The utility of gauging data from downstream locations will depend upon their distance from headwater sites, their position relative to reservoirs (where levels may reflect not solely precipitation, but recreational and socioeconomic use), and changes in watershed land cover. In addition, many gauges on intermediate size streams and rivers have been retired, and therefore problematic for developing stage relationships with headwater sites. However, long-term precipitation records may serve as surrogate for flow. The seasonal and interannual variation in precipitation and hydrologic observations provide the likelihood of flowing conditions. While year-round sampling (both dry and wet seasons) over several years may be optimal for categorizing or assessing a headwater site, researchers are rarely afforded such opportunities. For shorter-term studies, sampling should take place during the driest and wettest periods of the year to assess extreme conditions. If sampling is restricted to one visit, headwater index periods will typically be during the spring when flow is higher, and most aquatic organisms can be collected.

The gradual change in environmental conditions (e.g., lower dissolved oxygen, higher temperatures) as temporary habitats dry can be as critical to understanding mechanisms influencing biotic response as the duration and frequency of drying. Disturbances (disrupting force) or perturbations (sequence of disrupting force and system response) have been classified as either pulse or press events (Bender et al. 1984, Glasby and Underwood 1996). A pulse disturbance is characterized by a short and sharply delineated event (relative to the time scale of the response measure, Figure 2-1a), whereas a press disturbance has a continuous and constant level that is relative long-lasting (Figure 2-1b). In contrast to pulse and press disturbances, environmental conditions for many organisms worsen over time as streams dry (Slack and Feltz 1968, Towns 1985, Ostrand and Wilde 2004). Lake (2000, 2003) characterized this difference by conceptualizing that drying or drought was a "ramp" disturbance (Figure 2-1c). As the sequence of physicochemical changes progresses, greater stress is placed upon inhabitants, causing more taxa to succumb or emigrate over time. Rather than a steady sequence of physicochemical changes of a "ramp", Boulton (2003) argues that the sequence of changes may be better characterized as a series of "steps" (Figure 2-1d), wherein critical thresholds cause substantial shifts in wetted habitat (e.g., drying of riffles, subsurface habitat). Differences between the ramp and stepped models may be to some extent dependent upon the hierarchical scale through which the drying process is approached (Stanley et al. 1997). Some changes may be more apparent at small spatial or temporal scales, but undetectable at larger scales.

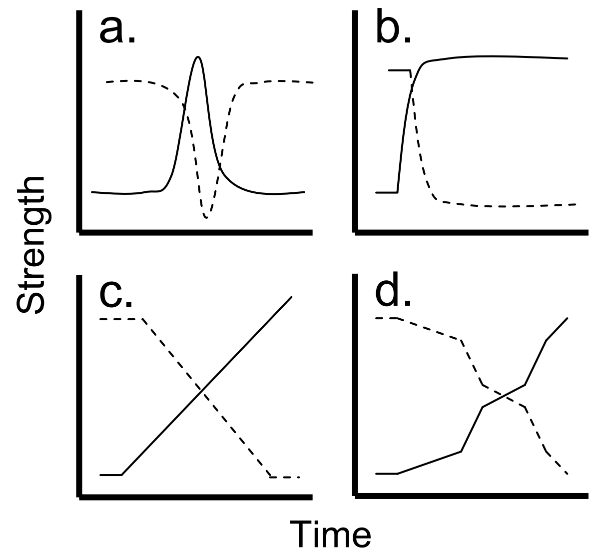

Figure 2-2 Types of disturbance (solid) and responses (dashed) in streams: pulse (a), press (b), ramp (c) and stepped (d). Based on figures from Lake (2003) and Boulton (2003).

Wetted area and volume are reduced initially in the drying sequence that leads to increased isolation of the wetted area from stream banks (contraction toward the deeper flowpaths in the channel) and between habitat units (contraction to pools). As discharge declines, flow may at first become braided between larger emergent substrates, then become limited to strong upwelling zones along the channel, and then finally cease altogether, leaving surface water to remain only in deep pools. These remaining pools shrink by evaporation and the hyporheic habitat (subsurface zone between the surface water and groundwater) dries if water deficit continues. The rate of channel drying varies with channel gradient, degree of exposure (to wind and sun), evapotranspiration by watershed vegetation, soil moisture status, and permeability or infiltration capacity from the surrounding watershed. Vegetation cover, type, and succession stage can also influence headwater stream hydrology (Bosch and Hewlett 1982). For example, annual stream flow is typically lower in streams draining conifers because of higher annual interception (and subsequently evaporation) of precipitation and higher transpiration loss at the beginning and end of the growing season than hardwoods (e.g., Swank et al. 1988). Streams draining limestone or "karst" geology retain surface water for shorter periods than streams draining geologic materials with lower hydraulic conductivity and effective porosity (e.g., sandstone and clay). Many of these factors will also influence the timing of flow commencement following precipitation (Blyth and Rodda 1973, Day 1978, de Vries 1995) or leaf abscission (Doyle 1991).

As previously mentioned, headwaters have a distinct bioassessment advantage because the small watershed areas make stressor identification more straight-forward. However, the timing of sample collection relative to the resumption of flow or start of drying is critical. The diversity, abundance, and biomass of benthic organisms increase and community composition shifts with time following the resumption of flow (Peterson 1987, Boulton and Lake 1992, Fritz and Dodds 2002, 2004). The rate of assemblage recovery varies with magnitude, duration, and extent of drying, particularly in relation to the permanence history (i.e., flow predictability) of streams. Resilience will likely vary among assemblage types and biological parameters (e.g., abundance, biomass) because of differences in the recovery mechanism (i.e., resistance vs. colonization), vagility, and growth rates. For purposes of bioassessments, samples should be collected near the peak of recovery from drying to maximize the number of indicator taxa present and biotic index or metric discrimination among condition categories.

Minimizing impacts associated with sampling
The potential for impacting streams during sampling is higher for headwater streams compared to larger streams and rivers, and therefore requires special consideration. Small wetted areas mean that sample collection and geomorphic measurements can potentially disturb a large portion of the local channel with potential adverse effects downstream. Individual substrates (e.g., cobble, small woody debris) that are inconsequential in larger streams and rivers may provide important geomorphic functions in headwater streams. Channel alteration caused by sampling may be more persistent in small streams than in larger channels because the power associated with flood events that resets channels is typically lower. Sampling in an upstream direction is typical in larger streams, and it is especially important when working in headwaters to minimize trampling the stream reach during assessments. Because headwater streams are small and positioned at the tips of stream networks, oversampling of unique populations and species is a concern. Headwater streams, particularly those that are spring-fed, often contain endemic taxa (Hubbs 1995, Ferrington 1995, Myers et al. 2001). Rather than further the endangerment of these unique communities, sampling protocols should provide information for their conservation.

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2.1 Study design for comparing across stream reaches with varying hydrologic permanence.

This section describes a specific study design used for comparing among headwater stream reaches varying in hydrologic permanence. The objectives were: 1) to characterize biological and physical features of reference headwater streams across a gradient of hydrologic permanence (frequency and duration of drying) and 2) to identify indicators of hydrologic permanence. The study focused on headwater streams in intact forests to limit potentially confounding effects of land use on hydrology. Streams were sampled in Indiana, Illinois, Kentucky, Ohio, New Hampshire, New York, Vermont, Washington, and West Virginia. The drainage area of study sites was restricted to basins ≤ 2.92 km^2 (1 mi^2) that corresponded to the upper boundary of streams measured. For assessment purposes, Ohio EPA is using this drainage area size to distinguish "Primary Headwater Habitat Streams" from the rest of the stream network (Ohio Environmental Protection Agency 2002).

Selecting study units that incorporate the range of hydrologic permanence (i.e., from ephemeral to perennial) was critical to meet the goals of the study. No data for annual hydrologic patterns were available prior to sampling, so the general positive relationship between drainage area and flow permanence was used to select sites (i.e., drainage area was used as a surrogate for flow permanence). As drainage area increases, groundwater storage increases and approaches the level of the streambed. Exceptions to this general pattern include perched aquifers and artesian springs in upper reaches that sustain year-round surface flow (Dunne and Leopold 1978) or substantial storage in swale soils above the channel head that sustains patches with perennial surface water (Hunter et al. 2005). These characteristics can result in fragmented longitudinal patterns of flow permanence along headwater streams (Lake 2003). Likewise, local changes in streambed topography along a stream influence the spatial pattern of hydrologic permanence. Sediments and woody debris originating from landslides, debris flows, and windthrow are transported downstream and deposited in reaches with lower gradient (Benda and Dunne 1987, Grizzel and Wolff 1998, Montgomery 1999). These deposits (i.e., sediment wedges) locally elevate the streambed above the dry season water table, causing reaches with such deposits to seasonally dry (May and Lee 2004, Harvey et al. 2005). Recognizing this, the study design incorporated multiple study units along multiple headwater streams. This design included a broad range of hydrologic regimes and capable of detecting repeating associations between stream features (biological and physical) and hydrology.

The study units were 30-m long reaches of stream channel (land form with bed and bank features). This length is on average 40X the headwater channel width and is consistent with study units used by USEPA in the Environmental Monitoring and Assessment Program (EMAP). Adjustment of the reach length may be needed to incorporate repeating geomorphic channel units. Three or four 30-m study reaches were selected within each stream. The aim was to include 1 reach with perennial flow, 2 reaches with varying degrees of intermittent flow and 1 reach with ephemeral flow. This design ensures sampling across a sufficient range of hydrologic conditions within a stream, while also allowing for multiple streams to be assessed. This sampling regime of the study required at least 2 sampling periods for each site within a year. These periods included visits in spring (wet season) and late summer (dry season), but not necessarily in that order. An initial visit to the streams during the dry season helps ensure that a perennial site is sampled, but it may be difficult to determine if a dry reach is intermittent or ephemeral at that time. Field visits during wet and dry seasons prior to selecting sites, where possible, may provide greater confidence in the distribution of sites across the flow permanence gradient.

Desktop selection procedure

In most cases the upstream study reaches along the streams were not marked with "blue lines", but appeared as "depressions" on 1:24 000 scale topographic maps (Figure 2-2). Red lines have been added to Figure 2-2 to show a more realistic and complete network of stream channels within the Falling Rock Branch watershed. The yellow line represents the approximate watershed boundary. Maps (typically 1:15 840 scale) published by USDA

NRCS (formerly Soil Conservation Service) provided better resolution of the headwater channel network, but still underestimated the extent of channels. Likewise, orthophotos (e.g., 1:12 000 scale) aided planning, but the ability to discern headwater channels varied with photo resolution and vegetation cover. Both types of maps and photos were used in the planning stage, but the topographic maps were more useful while in the field. The definition of the upstream extent of headwater channels is discussed in detail in Section 3.3.

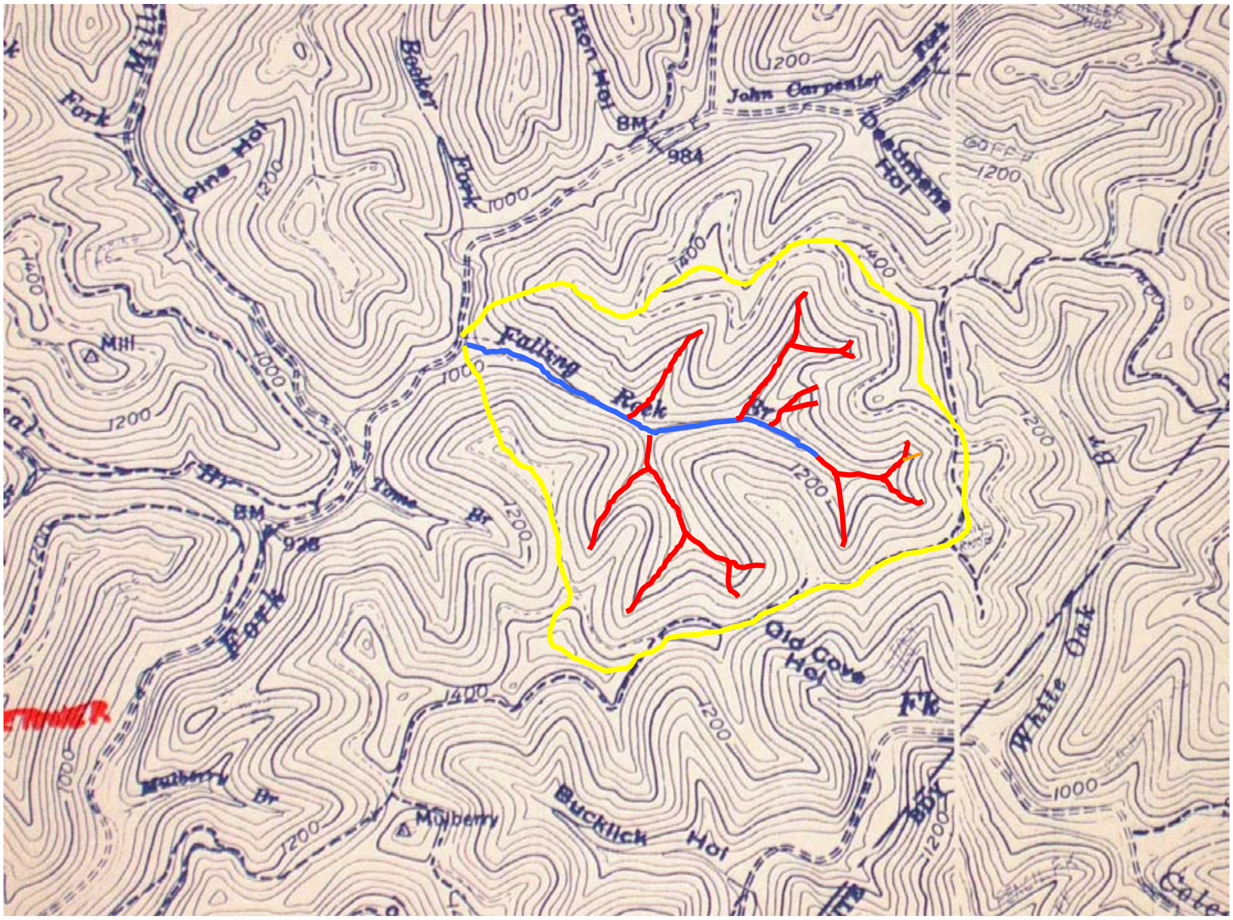

Figure 2-3 Map highlighting position of headwater channels within the watershed of Falling Rock Branch, KY. Yellow represents boundary of watershed, blue represents "blue line" designation on the 1:24 000 USGS topographic map (Noble 7.5 minute quadrangle, Breathitt County, KY), and red represent headwater channels not shown on the topographic map.

The channel network configuration, particularly dendritic or reticulate networks, creates a nonlinear relationship between distance downstream and drainage area. Drainage area does not increase gradually downstream, but rather increases in steps with each tributary confluence. This is an important consideration when selecting study reaches to maximize the range of hydrologic permanence. Tributary confluences are also useful landmarks for returning to study reaches for subsequent visits. Where possible, a more gradual drainage area transition between study reaches is preferred (Figure 2-

3). In some cases the entire drainage area of headwater stream may not be sufficient to supply perennial flow. In this situation a stream may need to be paired with an adjacent, larger tributary so there is a shared perennial site (Figure 2-4).

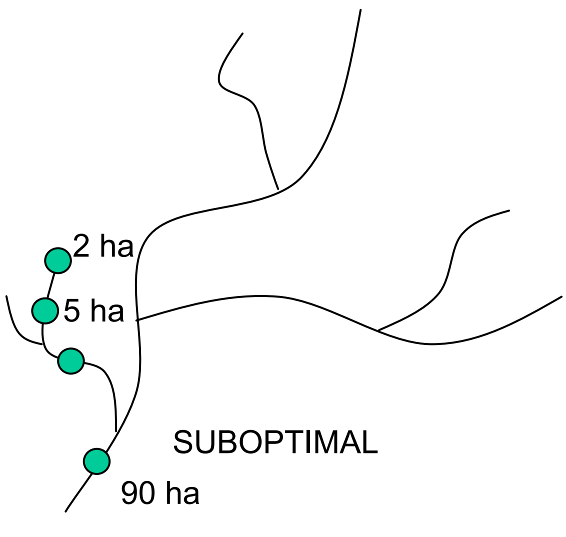

Figure 2-4 Schematic showing suboptimal and preferred longitudinal positioning of sites along headwater channels to maximize the range of hydrologic permanence across study sites. Hypothetical drainage areas are shown to further illustrate spatial hierarchy.

Properties that contained intact forest were identified and we obtained USGS 7.5 minute quadrangle maps (1:24 000 scale) for the selected areas. Land owners or managers of the properties were contacted. We described to them the objectives and design of the study and provided background material that they may need (e.g. Quality Assurance Project Plan, research proposal). We also inquired about headwater streams draining the property, especially pertaining to their flow permanence, current land use, ongoing or previous research downstream, and accessibility by roads and hiking trails. Being able to quickly travel between study reaches helped ensure that a sufficient number of study units were assessed and that sampling was done over a reasonable timeframe (i.e., within the same index period).

Field study reach – general selection guidelines

In the field we located the stream reaches preliminarily selected from the map. Final selection was adjusted to ensure that the study reaches were entirely upstream or downstream of tributary confluences. We also selected reaches having multiple habitat units (erosional and depositional habitats). Although large woody debris dams are characteristic features of intact forested streams, reaches with excessively large woody debris dams (prevented access to >50% of the wetted channel in the study reach) were avoided when possible. These structures are likely to 1) complicate the association between reach properties (physical and biological) and hydrology and 2) impede data collection. With this in mind, debris dams are common in some regions and will be unavoidable when designating study reaches.

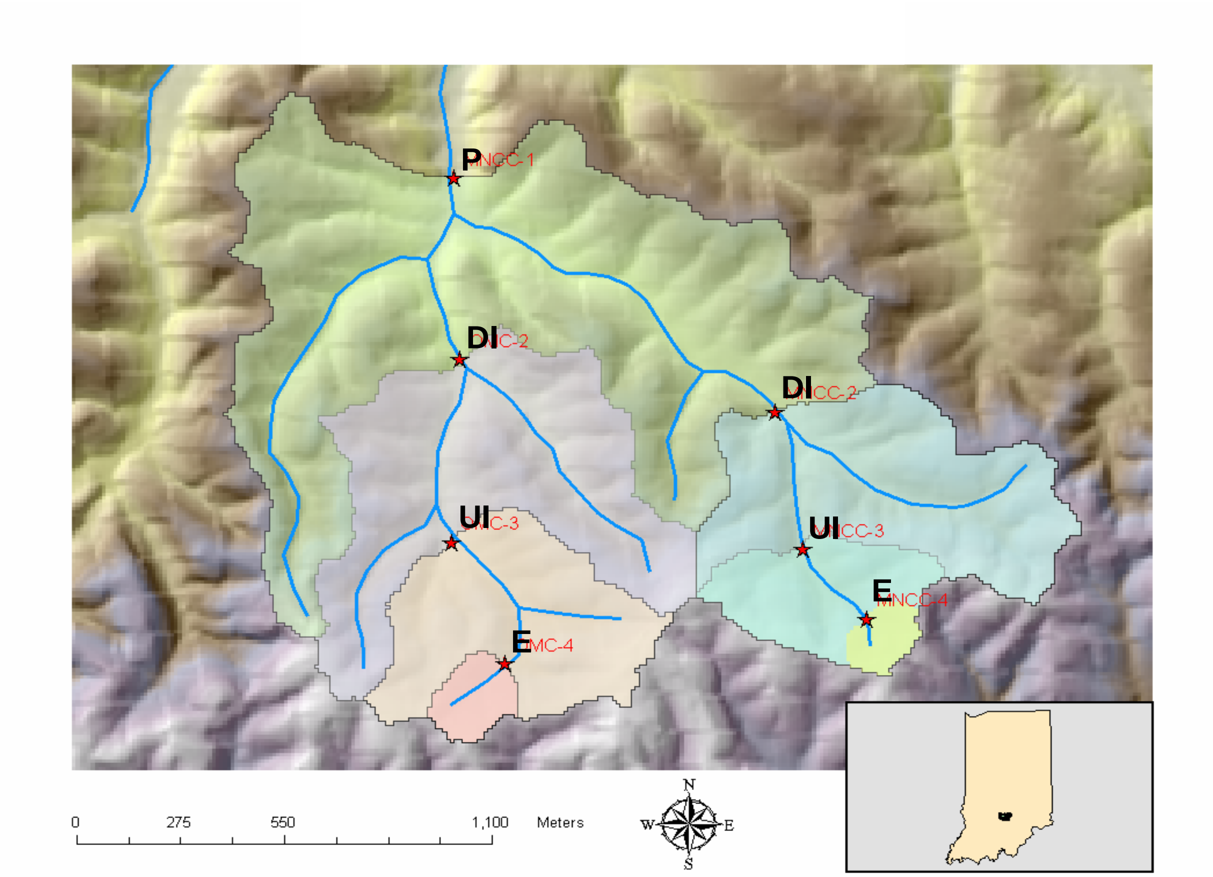

Figure 2-5 Map showing positioning of sites along two Indiana headwater streams where the downstream perennial site (P) is "shared" between two tributaries. DI = downstream intermittent; UI = upstream intermittent; and E = ephemeral. Shading shows cumulative drainage area in downstream direction.

Using a measuring tape, we marked the 30-m study reach from the downstream boundary (located at 0 m) to the upstream boundary (located at 30 m). The tape was positioned to follow the thalweg. The thalweg is the deepest flow path in a channel. The study reaches were designated using flagging tape or other clearly visible markers attached to trees near each boundary. The location of the study reach was identified on the topographic map or a PDA with electronic topographic maps, and a written description of the study reach location and appropriate locality information (e.g., topographic map, county, state) was entered on the field forms. Photographs of the study reach were taken and coordinates from a GPS unit were recorded. Study reaches were consistently identified by site numbers that increased in an upstream direction starting with 1 at the downstream-most reach (Figure 2-5).

Field selection – initial visit in spring (wet season)

When the initial field visit to a study region was in the spring (wet season), then sites were located as follows. The ephemeral site for each headwater stream studied was designated just upstream of the origin of intermittent flow (Paybins 2003; upstream-most location of spatially-continuous surface flow in the spring or wet season; Figure 2-5). The upstream intermittent site was positioned downstream of the origin of intermittent flow. The drainage area of the downstream intermittent site often incorporated at least an additional ephemeral drainage. Similarly, the perennial site frequently incorporated at least twice the drainage area of the downstream intermittent site (Figure 2-5).

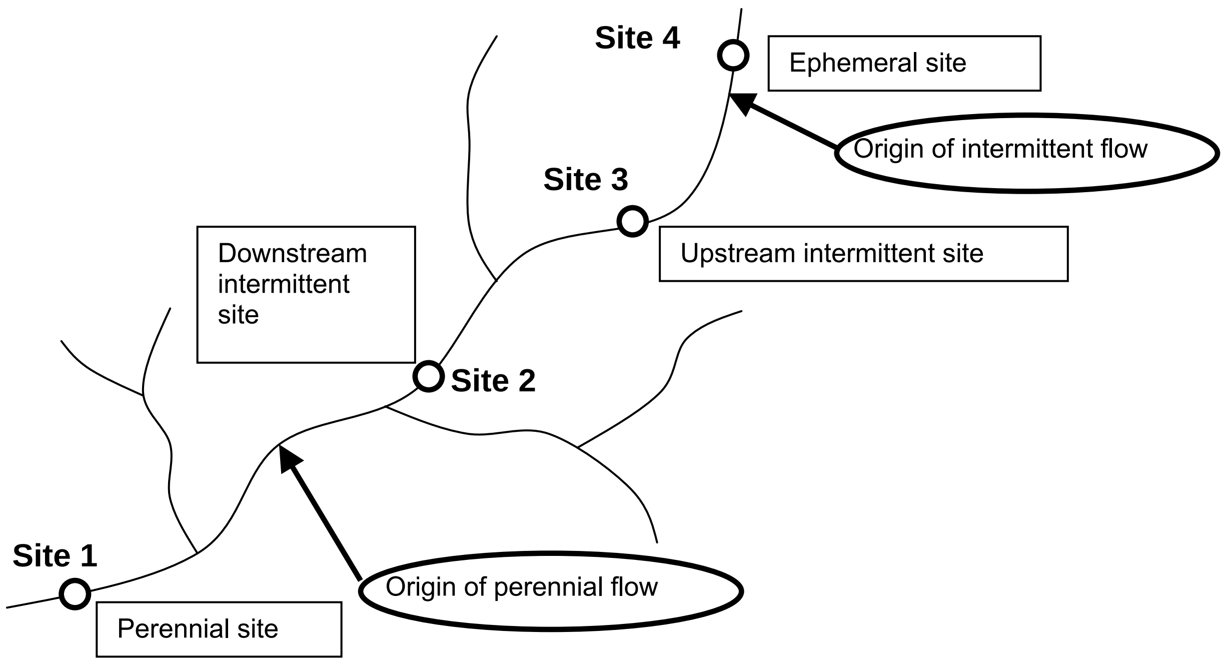

Figure 2-6 Schematic of headwater channels showing numerical designation and position of study sites relative to origins of intermittent and perennial flow.

The spatial pattern of hydrologic permanence may not reflect a downstream progression from ephemeral to intermittent to perennial reaches along headwater channels for reasons discussed earlier (e.g., perched aquifers, artesian springs). Incorporating multiple streams into the design may provide support for alternative longitudinal patterns of flow permanence within headwater drainages. Depending upon the precipitation and geographic setting the prevalence of some permanence categories and therefore variation in flow permanence among study sites is more subtle.

Field selection – initial visit in summer (dry season)

When the initial field visit to a study region was in the summer (dry season), then sites were located as follows. The origin of perennial flow (Paybins 2003; upstream-most location of spatially continuous surface flow in the summer or dry season; Figure 2-5) was located. The perennial site was positioned just downstream of the origin of perennial flow. The other three study reaches often did not have continuous surface flow during the summer. The next site upstream frequently drained approximately half the drainage area of the perennial site. The upstream intermittent site (Site 3) was often positioned at least one confluence upstream of Site 2. The ephemeral site was designated near the top of the watershed, but where there was a defined streambed and banks. Terrestrial herbaceous vegetation was common within the channel of the ephemeral study reach.

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Equipment and supplies

USGS 7.5 minute quadrangle map(s)
NRCS soil survey map(s)
Measuring tape (50 m)
Flagging or other marker
Camera
GPS unit or Handheld personal computer or Personal Digital Assistant (PDA) with digital maps and GPS card